Synopsis of the thesis entitled

Biological and Preliminary Phytochemical Evaluation of Three Folklore medicinal plants, *Melochia corchorifolia L, Chrozophora rottleri* (Geiseler) A. Juss. Ex Spreng and *Spilanthes acmella L*

By

MALLIKARJUNA RAO. TALLURI

M.Sc

Under the guidance of

(Guide)

Prof. Y. VENKATESWARA RAO

M.Sc., Ph.D.

Department of Botany,
College of Science and Technology,
Andhra University, Visakhapatnam
Andhra Pradesh, India

(Co-Guide)

Prof. B. GANGA RAO

M.Pharm., Ph.D. (India); Ph.D. (U.K.); P.D.F. (Australia)

College of Pharmaceutical Sciences
Andhra University, Visakhapatnam
Andhra Pradesh, India

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The thesis entitled "**Biological and Preliminary Phytochemical Evaluation of Three Folklore medicinal plants, *Melochia corchorifolia L, Chrozophora rottleri* (Geiseler) A. Juss. Ex Spreng and *Spilanthes acmella L*",** embodies the work on three important medicinal plants *M.corchorifolia*, *C.rottleri* and *S. acmella* are available in the state of Andhra Pradesh. In the traditional folklore practice these plants are used for treating diseases (*M. corchorifolia* for urinary disorders, abdominal swelling, dysentery, snakebites and sores; *C. rottleri* for jaundice, wounds healing and purifying blood; *S. acmella* for treatment of skin diseases, dysentery, bacterial and fungal skin diseases). However, no such report is available in the literature supporting its scientific validity. Hence, the present study was carried out to give scientific evidence to the folklore claims and support on the usage of these plants.

On the basis of the available information in literature, the natures of the chemical constituents were examined and the biological activities of the selected plants were evaluated after extracting with different solvents.

For a systematic representation of the work carried out, the thesis was presented in eight chapters.

Chapter-I: Introduction

An introduction to natural products as a source for drugs, the role of traditional medicine in the discovery of new drugs was dealt with in this chapter. The aims and objectives of the present study were given in detail.

Chapter-II: Review of Literature

This chapter deals with the morphological characters of the selected plants (*M. corchorifolia*, *C. rottleri* and *S. acmella*) with an updated review of phytochemical constituents and biological activities that have been dealt with by eminent scientists in this sphere are taken up in a detailed manner.

Chapter-III: PRELIMINARY PHYTOCHEMICAL SCREENING

This chapter deals with the preliminary phytochemical screening for the presence of various phytochemical constituents in the extracts of the selected plant and the quantification of total phenolic and alkaloid contents.

Qualitative and Quantitative phytochemical screening of *Melochia corchorifolia*:

Qualitative phytochemical screening the extracts of aerial part of *M. corchorifolia* revealed the presence of different phytochemical constituents like steroids, terpenoids, flavanoids, alkaloids, glycosides, phenols, tannins and carbohydrates. The extracts gave negative results for the presence of amino acids, oils, quinines and saponins.

The Quantified phenolic contents of the extracts of aerial part of *M. corchorifolia* were ranged from 16.28±0.52 to 34.22±0.43 (mg/gm). The methanol extract have more phenolic content i.e. 34.22±0.43 (mg/gm) than other extracts and the alkaloid content was ranging from 18.46±0.34 to 26.37±0.16 (mg/gm). The methanolic extract has more alkaloid content (26.37±0.16 mg/gm) than other extracts.

Qualitative and Quantitative phytochemical screening of *Chrozophora rottleri*:

Qualitative phytochemical screening the extracts of *C. rottleri* revealed the presence of different phytochemical constituents like steroids, terpenoids, flavanoids, alkaloids, glycosides, phenols, tannins and carbohydrates. The extracts gave negative results for the amino acids, oils, quinines and saponins.

The Quantified phenolic contents of the extracts of *C.rottleri* were ranged from 10.41±0.62 to 41.36±0.54 (mg/gm). The methanol extract have more phenolic content i.e. 41.36±0.54 (mg/gm) than other extracts and the alkaloid content was ranging from 13.4±0.18 to 39.62±0.48 (mg/gm). The methanolic extract has more alkaloid content (39.62±0.48 mg/gm) than other extracts.

Qualitative and Quantitative phytochemical screening of *Spilanthes acmella:*

Qualitative phytochemical screening the extracts of aerial parts of *S.acmella* revealed the presence of different phytochemical constituents like steroids, terpenoids, flavanoids, alkaloids, glycosides, tannins, carbohydrates, oils and amino acids. The extracts gave negative results for the presence of quinines and saponins.

The Quantified phenolic contents of the extracts of aerial parts *S. acmella* were ranged from 13.52±0.19 to 38.83±0.68 (mg/gm). The methanol extract have more phenolic content i.e. 38.83±0.68 (mg/gm) than other extracts and the alkaloid content was ranging from 17.73±0.38 to 32.64±0.86 (mg/gm). The methanolic extract has more alkaloid content (26.37±0.16 mg/gm) than other extracts.

Chapter-IV: ACUTE TOXICITY STUDIES

Acute oral toxicity studies in mice of either sex (20-30 g) revealed that the extracts up to 2000 mg/kg have not produced any mortality in experimental animals.

Chapter-V: *IN VITRO* ANTIOXIDANT ACTIVITY:

This chapter deals with the introduction to free radicals, antioxidants and list of plants with antioxidant activity. The experimental procedures for *In vitro* anti oxidant activity of the extracts of the aerial parts of *M.corchorifolia,* whole plant of *C. rottleri,* aerial parts of *S. acmella* and known antioxidant ascorbic acid as standard the free radicals superoxide, hydroxyl, DPPH were tested and their results discussed in an elaborate manner.

A) *In vitro* antioxidant activity on Superoxide Radical

Melochia corchorifolia

In the present study, hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *M.corchorifolia* were found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin. The mean IC_{50} values for superoxide radical of Hydro-alcoholic, methanol, ethyl acetate and hexane extracts were found to be 206µg, 127µg, 530µg and 901µg respectively. The mean IC_{50} value of ascorbic acid was found to be 59.3µg.

Chrozophora rottleri

In the present study, hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *C. rottleri* were found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin. The mean IC_{50} values for superoxide radical of hydroalcoholic, methanolic, ethyl acetate and hexane extracts were found to be 254µg, 155µg, 350µg and 537µg respectively. The mean IC_{50} value of ascorbic acid was found to be 59.3µg.

Spilanthes acmella

In the present study, hydroalcoholic, methanol, ethyl acetate and hexane extracts of *S.acmella* were found to possess concentration dependent scavenging activity on superoxide generated by photoreduction of riboflavin. The mean IC_{50} values for superoxide radical of hydroalcoholic, methanol, ethyl acetate and hexane extracts were

found to be 271µg, 189µg, 226µg and 503µg respectively. The mean IC_{50} value of ascorbic acid was found to be 59.3µg.

B) *In vitro* antioxidant activity on Hydroxyl Radical

Melochia corchorifolia

The hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *M.corchorifolia* were found to possess concentration dependent scavenging activity on hydroxyl radicals. The mean IC_{50} values for hydroxyl radical of Hydro-alcoholic, methanol, ethyl acetate and hexane extracts were found to be 384µg, 240µg, 490µg and 501µg respectively. The mean IC_{50} value of ascorbic acid was found to be 66µg.

Chrozophora rottleri

The hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *C. rottleri* were found to possess concentration dependent scavenging activity on hydroxyl radicals. The mean IC_{50} values for hydroxyl radical of hydroalcoholic, methanolic, ethyl acetate and hexane extracts were found to be 232µg, 181µg, 278µg and 477µg respectively. The mean IC_{50} value of ascorbic acid was found to be 66µg.

Spilanthes acmella

The hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *S.acmella* were found to possess concentration dependent scavenging activity on hydroxyl radicals. The mean IC_{50} values for hydroxyl radical of hydroalcoholic, methanolic, ethyl acetate and hexane extracts were found to be 204µg, 154µg, 225µg and 485µg respectively. The mean IC_{50} value of ascorbic acid was found to be 66µg.

C) *In vitro* antioxidant activity on DPPH Radical

Melochia corchorifolia

The hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *M.corchorifolia* were found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC_{50} values for DPPH radical of hydroalcoholic, methanolic, ethyl acetate and hexane extracts were found to be 286µg, 179µg, 470µg and 971µg respectively. The mean IC_{50} value of ascorbic acid was found to be 16µg.

Chrozophora rottleri

The hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *C. rottleri* were found to possess concentration dependent scavenging activity on DPPH radicals.

The mean IC_{50} values for DPPH radical of hydroalcoholic, methanolic, ethyl acetate and hexane extracts were found to be 201µg, 166µg, 281µg and 495µg respectively. The mean IC_{50} value of ascorbic acid was found to be 16µg.

Spilanthes acmella

The hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *S.acmella* were found to possess concentration dependent scavenging activity on DPPH radicals. The mean IC_{50} values for DPPH radical of hydroalcoholic, methanolic, ethyl acetate and hexane extracts of *S.acmella* were found to be 226µg, 313µg, 377µg and 546µg respectively. The mean IC_{50} value of ascorbic acid was found to be 16µg.

Among the four extracts of *M. corchorifolia*, the methanolic extract showed better activity than other extracts on the tested three free radicals. The order of activity in the following manner: Ascorbic acid >methanol extract >Hydro-alcoholic extract > ethyl acetate extract> hexane extract.

Among the four extracts of *C. rottleri*, the methanolic extract showed better activity than other extracts on the superoxide and hydroxyl free radicals. The Hydro-alcoholic extract showed better activity on DPPH free radical than other extracts. The order of activity in the following manner: Ascorbic acid >methanol extract >Hydro-alcoholic extract > ethyl acetate extract> hexane extract.

Among the four extracts of *S.acmella*, the methanolic extract showed better activity than other extracts on the superoxide and hydroxyl free radicals. The Hydro-alcoholic extract showed better activity on DPPH free radical than other extracts. The order of activity in the following manner: Ascorbic acid >methanolic extract >hydroalcoholic extract > ethyl acetate extract> hexane extract.

Chapter-VI: HEPATOPROTECTIVE ACTIVITY:

This chapter deals with the introduction to liver, liver diseases, role of herbal medicine in liver diseases and the hepatoprotective activity of selected plants against CCl_4-induced hepatotoxicity.

Evaluation of effect of plant extracts of *Melochia corchorifolia* on CCl_4 induced liver toxicity in rats*:*

Hydroalcoholic (ethanol70%), Methanolic, Ethyl Acetate and Hexane extracts were tested at doses of 125mg/kg, 250mg/kg and 500mg/kg. The percentage protection

produced by the standard and extracts of *M.corchorifolia* were calculated based on SGOT, SGPT, ALP and total serum bilirubin levels on 7th day of experiment in each case.

Group I was treated with vehicle showed no significant change in the biomarkers of liver enzymes (SGOT, SGPT, ALP and total serum bilurubin) levels. Group II was treated with CCl_4, There is increase in SGOT, SGPTT, ALP, total serum bilurubin levels.

Group III was treated with Silymarin, at a dose of 50mg/kg and after one hour followed by CCl_4 intoxication, produces increase in SGOT, SGPT, ALP and total serum bilirubin levels and the percentage protection offered by the silymarin against the increase in SGOT, SGPT, ALP, and total serum bilurubin levels 93.55%, 94.32%, 89.04% and 80% respectively.

Groups IV, V and VI were treated with hydroalcoholic extract of *M. corchorifolia* orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 31.87%, 37.65%, 57.48% and 40.0%, 52.30%, 48.24%, 69.29%, and 60.0%, 74.91%, 70.24%, 78.46% and 80.00% respectively.

Groups VII, VIII and IX Received *M. corchorifolia* Methanolic extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 44.65%, 40.06%, 57.95% and 50.0%, 57.86%, 56.47%, 64.65% and 70.00%, 78.98%, 79.65%, 82.48% and 80.00% respectively.

Groups X, XI and XII received *M. corchorifolia* Ethyl acetate extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 27.13%,

27.41%, 54.41% and 50.0%, 44.14%, 50.35%, 62.52% and 60.00%, 60.75%, 66.82%, 74.88% and 70.00% respectively.

Groups XIII, XIV and XV received *M. corchorifolia* Hexane extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 21.42%, 22.11%, 50.5% and 40.00%, 39.02%, 36.04%, 55.98% and 50.00%, 53.42%, 50.23%, 70.19% and 70.00% respectively.

The order of hepatoprotective activity of *M. corchorifolia* based on SGPT (ALT) levels is as follows:

Silymarin (50mg/kg)(93.55%) > Methanolic extract (500mg/kg)(79.65%) > Hydro-alcoholic extract (500mg/kg)(70.24%) > Ethyl acetate extract (500mg/kg)(66.82%) > Hexane extract (500mg/kg)(50.23%).

Evaluation of effect of *Chrozophora rottleri* plant extracts on CCl_4 induced liver toxicity in rats

Hydroalcoholic (ethanol70%), Methanol, Ethyl Acetate and Hexane extracts were tested at doses of 125mg/kg, 250mg/kg and 500mg/kg. The percentage protection produced by the standard and extracts of *C. rottleri* were calculated based on SGOT, SGPT, ALP and total serum bilirubin levels on 7^{th} day of experiment was calculated in each case.

Group I was treated with drug vehicle showed no significant change in the biomarkers of liver enzymes (SGOT, SGPT, ALP and total serum bilirubin). Group II was treated with CCl_4, There is increase in SGOT, SGPTT, ALP, total serum bilurubin levels.

Group III received Silymarin, at a dose of 50mg/kg and after one hour followed by CCl_4 intoxication, produces increase in SGOT, SGPT, ALP and total serum bilirubin levels and the percentage protection offered by the sylmarin against the increase in SGOT, SGPT, ALP, and total seru bilurubin levels 93.55%, 94.32%, 89.04% and 80% respectively.

Groups XVI, XVII and XVIII received Hydroalcoholic extract of *C.rottleri* orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 36.41%, 32.35%, 55.34% and 60.00%, 45.75%, 48.19%, 63.77%, and 70.00%, 52.71%, 56.33%, 68.08% and 80.00% respectively.

Groups XIX, XX and XXI Received *C. rottleri* Methanolic extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 42.56%, 38.98%, 58.22% and 60.00%, 52.05%, 54.88%, 66.26% and 70.00%, 59.19%, 60.52%, 66.68% and 80.00% respectively.

Groups XXII, XXIII and XXIV received *C. rottleri* Ethyl acetate extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 30.23%, 29.24%, 51.57% and 50.00%, 40.10%, 40.93%, 59.86% and 60.00%, 52.48%, 55.99%, 61.08% and 70.00% respectively.

Groups XXV, XXVI and XXVII received *C. rottleri* Hexane extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication produced mild increase in SGOT, SGPT, ALP and total serum bilirubin levels. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 18.70%, 18.93%, 48.14% and 50.00%, 32.52%, 33.41%, 54.81% and 60.00%, 50.88%, 54.28%, 58.64% and 70.00% respectively.

The order of hepatoprotective activity of *C. rottleri* based on SGPT (ALT) levels is as follows:

Silymarin (50mg/kg)(93.55%) > Methanolic extract (500mg/kg)(78.73%) > Hydroalcoholic extract (500mg/kg)(60.52%) > Ethyl acetate extract (500mg/kg)(56.33%) > Hexane extract (500mg/kg)(45.99%).

Evaluation of effect of *Spilanthes acmella* plant extracts on CCl_4 induced liver toxicity in rats

Hydroalcoholic (ethanol70%), Methanolic, Ethyl Acetate and hexane extracts were tested at doses of 125mg/kg, 250mg/kg and 500mg/kg. The percentage protection produced by the standard and extracts of *S. acmella* was were calculated based on SGOT, SGPT, ALP and total serum bilirubin levels on 7^{th} day of experiment was calculated in each case.

Group I was treated with drug vehicle showed no significant change in the biomarkers of liver enzymes (SGOT, SGPT, ALP and total serum bilurubin). Group II was treated with CCl_4, There is increase in SGOT, SGPTT, ALP, total serum bilurubin levels.

Group III was treated with Silymarin, at a dose of 50mg/kg and after one hour followed by CCl_4 intoxication, produces increase in SGOT, SGPT, ALP and total serum bilirubin levels and the percentage protection offered by the sylmarin against the increase in SGOT, SGPT, ALP, and total seru bilurubin levels 93.55%, 94.32%, 89.04% and 80% respectively.

Groups XXVIII, XXIX and XXX were treated with hydroalcoholic extract of *S. acmella* orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 43.10%, 30.92%, 57.23% and 60.00%, 55.80%, 42.87%, 66.94%, and 70.00%, 74.53%, 70.51%, 72.25% and 80.00% respectively.

Groups XXXI, XXXII and XXXIII were treated with *S. acmella* methanolic extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels

were 47.81%, 36.52%, 61.10% and 60.00%, 55.17%, 55.22%, 69.94% and 70.00%, 84.39%, 79.04%, 78.15% and 80.00% respectively.

Groups XXXIV, XXXV and XXXVI were treated with *S. acmella* ethyl acetate extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 29.33%, 22.02%, 56.67% and 60.00%, 49.89%, 45.66%, 61.83% and 70.00%, 64.01%, 64.82%, 64.58% and 80.00% respectively.

Groups XXXVII, XXXVIII and XXXIX were treated with *S. acmella* Hexane extract orally at doses of 125mg/kg, 250mg/kg and 500 mg/kg and after one hour followed by CCl_4 intoxication, enzymes (SGOT, SGPT, ALP and total bilirubin) levels produced due to the effect of silymarin and extracts. The percentage protection produced by the extracts on the reduction of SGOT, SGPT, ALP and total serum bilirubin levels were 18.68%, 17.45%, 50.01% and 40.00%, 40.27%, 35.29%, 58.18% and 60.00%, 53.28%, 48.75%, 63.97% and 70.00% respectively.

The order of hepatoprotective activity of *S. acmella* based on SGPT (ALT) levels is as follows:

Silymarin (50mg/kg)(93.55%) > Methanolic extract (500mg/kg)(79.04%) > Hydro-alcoholic extract (500mg/kg)(70.51%) > Ethyl acetate extract (500mg/kg)(64.82%) > Hexane extract (500mg/kg)(48.75%).

Chapter-VII: ANTIINFLAMMATORY ACTIVITY:

This chapter describes the anti-inflammatory activity of plants i.e. *Melochia corchorifolia, Chrozophora rottleri* and *Spilanthes acmella* extracts in carrageenan induced paw ooedema in rats.

Methanolic extracts have produced maximum activity than hydroalcoholic, ethyl acetate and hexane extracts. The results were expressed as maximal paw oedema (maximal peak during the 6 h) and as total paw oedema (area under the time-course curve) and presented as Mean ± S.E.M., n=6.

Effect of *Melochia corchorifolia* plant extracts on carrageenan-induced rat paw oedema

Indomethacin and hydroalcoholic (Ethanol 70 %) extract of aerial parts of *M.corchorifolia* at 125 mg/kg, 250 mg/kg and 500mg/kg doses significantly inhibited the maximal paw oedema response by 68.26±0.63, 19.69±3.35, 39.31±3.41 and 58.80±3.65 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 28.93±2.58, 45.57±4.92 and 59.10±2.78 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and methanolic extracts of aerial parts of *M.corchorifolia* at 125 mg/kg, 250 mg/kg and 500mg/kg doses significantly inhibited the maximal paw oedema response by 68.26±0.63, 17.95±3.04, 29.37±3.47 and 56.36±1.73 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 38.14±3.43, 50.91±3.65 and 54.97±1.95 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and ethyl acetate extract of aerial parts of *M.corchorifolia* significantly inhibited the maximal paw oedema response by 68.26±0.63, 16.69±2.42, 26.11±2.86 and 45.89±3.23 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 32.10±2.96, 41.76±3.49 and 56.04±1.86 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and hexane extract of aerial parts of *M.corchorifolia* significantly inhibited the maximal paw oedema response by 68.26±0.63, 13.96±3.42, 25.15±2.53 and 46.27±1.32 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 64.92±0.95, 29.54±2.32, 16.52±1.93 and 42.10±3.50 respectively over 6 h when compared to the control group treated with drug vehicle.

Effect of *Chrozophora rottleri* plant extracts on carrageenan-induced rat paw oedema

Indomethacin and hydroalcoholic (Ethanol 70 %) extract of *C. rottleri* at 125 mg/kg, 250 mg/kg and 500mg/kg doses significantly inhibited the maximal paw oedema

response by 68.26±0.63, 15.30±1.82, 27.38±1.16 and 49.65±1.88 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.02, 25.30±2.19, 31.56±2.28 and 48.90±2.20 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and methanolic extract of *C. rottleri* at 125 mg/kg, 250 mg/kg and 500mg/kg doses significantly inhibited the maximal paw oedema response by 68.26±0.63, 15.03±4.06, 29.07±0.39 and 53.47±2.19 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 27.13±4.27, 39.06±4.79 and 52.46±3.24 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and ethyl acetate extract of *C. rottleri* significantly inhibited the maximal paw oedema response by 68.26±0.63, 13.94±3.54, 24.63±3.57 and 41.17±2.00 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 22.06±3.80, 35.49±3.74 and 46.10±2.10 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and hexane extract of *C. rottleri* significantly inhibited the maximal paw oedema response by 68.26±0.63, 12.72±2.73, 21.21±2.85 and 38.93±1.72 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 19.02±1.71, 32.04±2.08 and 44.60±3.65 respectively over 6 h when compared to the control group treated with drug vehicle.

Effect of *Spilanthes acmella* plant extracts on carrageenan-induced rat paw oedema

Indomethacin and hydroalcoholic (Ethanol 70 %) extract of aerial parts of *S. acmella* at 125 mg/kg, 250 mg/kg and 500mg/kg doses significantly inhibited the maximal paw oedema response by 68.26±0.63, 15.33±3.37, 28.59±2.54 and 51.28±1.67 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 21.29±3.59, 31.86±3.52 and 52.34±1.84 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and methanolic extract of aerial parts of *S. acmella* at 125 mg/kg, 250 mg/kg and 500mg/kg doses significantly inhibited the maximal paw oedema response by 68.26±0.63, 18.05±2.92, 30.68±1.63 and 56.49±1.34 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 17.39±2.56, 29.35±1.81 and 46.17±2.59 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and ethyl acetate extract of aerial parts of *S. acmella* significantly inhibited the maximal paw oedema response by 68.26±0.63, 14.09±1.83, 24.5±3.00 and 45.95±1.86 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 17.39±2.56, 29.35±1.81 and 46.17±2.59 respectively over 6 h when compared to the control group treated with drug vehicle.

Indomethacin and hexane extract of aerial parts of *S. acmella* significantly inhibited the maximal paw oedema response by 68.26±0.62, 12.60±1.52, 24.5±2.30 and 42.10±2.33 respectively during the 6 h of the carrageenan-induced rat paw acute inflammation. The total oedema response (AUC) was inhibited by 67.42±1.05, 17.39±2.46, 29.35±1.81 and 46.17±2.59 respectively over 6 h when compared to the control group treated with drug vehicle.

The results suggested that the standard drug Indomethacin and the tested selected plant extracts (*M. corchorifolia, C. rotteleri and S.s acmella*) were also significantly inhibited paw oedema. Among all extracts methanol and hydro alcoholic extracts of selected three plants produced significant effect, ethyl acetate and hexane extracts produced moderate percentage inhibition produced low level of percentage protection in reducing paw oedema. In all extracts methanolic extract of the aerial parts *M. corchorifolia* at a dose of 500mg/kg showed more percentage of inhibition and hexane extract of *C. rotteleri* at a dose of 125mg/kg showed low percentage inhibition in reduction of paw oedema against carrageen induced rats.

Chapter-VIII: ANTIBACTERIAL ACTIVITY:

This chapter deals with the plant extracts that were screened for antibacterial activity against various Gram +ve bacteria and Gram –ve bacteria. Antibacterial screening of the plant extracts was carried out by the cup plate method.

Antibacterial activity of *Melochia corchorifolia*

Hexane extract produced low zones of inhibition against bacterial strains compared with other extracts and it had not shown zone of inhibition against *Streptococcus faecalis, Staphylococcus epidermidis, Escherichia coli* and *Pseudomonas aeruginosa* at a concentration of 50µg/cup. Hexane extract showed highest zone of inhibition (12mm) against *Staphylococcus aureaus* and *Klebsiella pneumoniae* at a concentration of 400µg/cup.

Ethanol (70%) and Ethyl Acetate extracts showed moderate zones of inhibition on tested bacterial strains. Ethanol extract showed maximum zone of inhibition (18mm) over *Pseudomonas aeruginosa* at a concentration of 400µg/cup. Ethyl Acetate extract had not show zone of inhibition against *Streptococcus faecalis* at a dose of 50µg/cup and showed maximum zone of inhibition (15mm) on *Klebsiella pneumoniae*.

The methanol extract showed better activity against tested bacterial strains compared to other extracts. Methanol extract shown maximum zones of inhibition (19mm, 18mm and 18mm) over *Pseudomonas aeruginosa, Bacillus megaterium* and *Klebsiella pneumoniae* at a concentration of 400µg/cup.

Antibacterial activity of *Chrozophora rottleri*

Hexane extract produced low zones of inhibition against bacterial strains compared with other extracts. Hexane extract had not show zone of inhibition against Gram +ve and gram –ve bacterial strains except *Pseudomonas aeruginosa* at a concentration of 50µg/cup. Hexane extract shown highest zone of inhibition (11mm) against gram –ve bacterial strains except *Salmonella typhimurium* (10mm) at a concentration of 400µg/cup.

Ethanol (70%) and Ethyl Acetate extracts showed moderate zones of inhibition on tested bacterial strains. Ethanol extract shown maximum zone of inhibition (16mm) over *Bacillus megaterium* and *Klebsiella pneumoniae* at a concentration of 400µg/cup. Ethyl Acetate extract had not show zone of inhibition against *Streptococcus faecalis* and *Staphylococcus aureaus* at a concentration of 50µg/cup and showed maximum zone of inhibition (14mm) over *Pseudomonas aeruginosa* at a concentration of 400µg/cup.

The methanol extract showed better activity against tested bacterial strains compared to other extracts. Methanol extract showed maximum zones of inhibition (18mm) on *Klebsiella pneumoniae* at a concentration of 400µg/cup.

Antibacterial activity of *Spilanthes acmella*

Hexane extract produced low zones of inhibition against bacterial strains compared with other extracts. Hexane extract had not shown zone of inhibition against *Streptococcus faecalis, Staphylococcus epidermidis* (Gram +ve) and gram –ve bacterial strains except *Klebsiella pneumoniae* at a concentration of 50µg/cup. Hexane extract showed highest zone of inhibition (13mm) against *Klebsiella pneumoniae* at a concentration of 400µg/cup.

Ethanol (70%) and Ethyl Acetate extracts showed moderate zones of inhibition on tested bacterial strains. Ethanol extract shown maximum zone of inhibition (16mm) over *Staphylococcus epidermidis* and *Salmonella typhimurium* at a concentration of 400µg/cup. Ethyl Acetate extract had not shown zone of inhibition against *Streptococcus faecalis* at a concentration of 50µg/cup and showed maximum zone of inhibition (15mm) on *Klebsiella pneumoniae* at a concentration of 400µg/cup.

The methanol extract showed better activity against tested bacterial strains compared to other extracts. Methanol extract shown maximum zones of inhibition (19mm and 18 mm) on *Pseudomonas aeruginosa, Staphylococcus epidermidis* and *Klebsiella pneumoniae* at a concentration of 400µg/cup.

The results in this chapter, the selected plant extracts at a concentrations of 50 µg, 100 µg, 200 and 400 µg per each cup exhibited considerable antibacterial activity against tested bacterial species (gram +ve and gram –ve). Among all extracts selected plants methanolic extracts of shown better antibacterial activity against gram –ve bacteria than gram +ve bacteria.

On the basis of the results in the present study, it is concluded that the selected plants *M.corchorifolia, C. rottleri* and *S. acmella* are possessed potential biological activities and the study scientifically justifies their use in the folklore remedies. The isolation and characterization of active constituent(s) responsible for the biological activities worth study further.